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Characterisation of PPNG and non-PPNG *Neisseria gonorrhoeae* isolates from Singapore

C L Poh, J C Ocampo, E H Sng, S M Bygdeman

Abstract

***Objective*—To characterise *Neisseria gonorrhoeae* isolates from Singapore.**

***Design*—Characterisation of *Neisseria gonorrhoeae* isolates by auxotyping, serological analysis and plasmid profile analysis.**

***Specimens*—Sixty randomly collected isolates from 41 symptomatic, untreated males and 19 female prostitutes were studied.**

***Results*—Auxotyping of 25 PPNG and 35 non-PPNG strains showed that the Pro⁻ auxotype was prevalent among both PPNG (56%) and non-PPNG (42·5%) strains. Prototrophic strains comprised 28% of PPNG and 32·5% of non-PPNG strains respectively. Serovar analysis showed that with the exception of seven serogroup WI strains, the majority belonged to serogroup WII/III. Serovar Aedih was predominant among both serogroup WI PPNG (80%) and non-PPNG (100%) strains. Serogroup WII/III PPNG strains were represented by nine serovars with the predominant serovars being Bacjk (28%) and Bcgjk (16%). Eleven serovars were identified in the WII/III non-PPNG strains and the major serovars were Bajk (20%), Bacjk (17%), Back (11·4%) and Beghjk (11·4%). Analysis of the 25 PPNG strains showed that 16 of them carried the 4·4 MDa (Asian type) resistance plasmid and nine strains harboured the 4·4 MDa plasmid in conjunction with the 24·5 MDa transfer plasmid. The cryptic plasmid of 2·6 MDa was present in 27 of the 35 non-PPNG strains. Five of the non-PPNG strains harbouring the cryptic plasmid also contained the 24·5 MDa transfer plasmid. The plasmid combination of 2·6 + 7·8 + 24·5 MDa was detected in three non-PPNG strains.**

***Conclusion*—The combination of epidemiological methods used in this study indicated the heterogeneity of *N gonorrhoeae* strains in Singapore. A total of 16 different combinations of auxotype, plasmid profile and serovar were seen in the 25 PPNG strains compared with 24 such combinations in the 35 non-PPNG strains. Such sensitive differentiation would otherwise not be possible using either auxotype-serovar (A/S) or auxotype-plasmid analysis.**

Introduction

Monitoring the international dissemination of gonococcal infections will require epidemiological characterisation of *Neisseria gonorrhoeae* isolates from various countries. Epidemiological data regarding the phenotypes of *N gonorrhoeae* are now available from countries such as Africa, Britain, Canada, Greece, Holland, Norway, Spain, Sweden and the United States.[1-9] Strains from these countries have been extensively characterised by auxotyping, serological classification and plasmid profile analysis. With the exception of a previous study by Odugbemi *et al* (1983)[13] of strains from Korea, few strains from South East Asia have been characterised to such detail.[3 10] As countries in South East Asia form important foci for the transmission of PPNG (Asian⁺ strains) to the rest of the world, it is essential to characterise the gonococcal populations from South East Asian countries in terms of the variables that have been used to characterise strains from the rest of the world.

The present study was undertaken to define the *N gonorrhoeae* population in Singapore by auxotyping, serological classification and plasmid profile analysis.

Materials and methods

Gonococcal strains

We studied 60 *N gonorrhoeae* strains randomly isolated over 6 months in 1984 from both male and female patients who attended the Middle Road Hospital as outpatients. Twenty seven of the isolates

Department of Microbiology, Faculty of Medicine, National University of Singapore, Lower Kent Ridge Road, Singapore 0511
C L Poh, J C Ocampo

Pathology Department, Singapore General Hospital Outram Road, Singapore 0316
E H Sng

Department of Clinical Bacteriology, Huddinge University Hospital, Karolinska Institute, Stockholm, Sweden
S M Bygdeman

were from symptomatic, untreated male patients with urethritis while the rest were from female prostitutes. All isolates were confirmed as *N gonorrhoeae* by colony morphology, Gram stain, oxidase reaction and acid production from glucose but not maltose, sucrose, lactose or fructose. Purified stock cultures were maintained in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md) containing 20% glycerol and stored at −70°C. Isolates were subcultured on modified Thayer-Martin agar (MTM, BBL) and incubated at 36°C with a 5% CO_2 atmosphere in a humidified incubator for 20 hours. The isolates were tested for beta-lactamase production by means of the chromogenic cephalosporin substrate, Nitrocefin (Oxoid).

Serological characterisation

The strains were serologically characterised by coagglutination using monoclonal antibodies to protein I as described by Bygdeman *et al.*[10] Isolates were identified as belonging to serogroups designated WI or WII/WIII. Serogroups were subdivided into different serovars using monoclonal antibodies specific for protein IA or IB. For the identification of WI strains, seven protein IA-specific monoclonal antibodies were used. Protein IA-specific antibodies were designated as follows: 4A12,b; 2F12,d; 6G9,f; 6D9,g; 5D1,h; 5G9,i; and 5C2,k. Identification of WII and WIII strains was achieved by using eight protein IB-specific monoclonal antibodies. Protein IB-specific antibodies were designated as follows: 3C8,a; 1F5,b; 2D6,c; 2H7,e; 2G2,g; 2D4,h; 3B10,j; and 2H1,k. All the designations of the WI serovars begin with an A (protein IA) and the designations of the WII and WIII serovars begin with B (protein IB). All isolates were typed at the Karolinska Institute.

Auxotyping

Auxotyping was performed as described by Catlin.[11] Strains were tested for their requirement for proline (Pro^-), arginine (Arg^-), methionine (Met^-), hypoxanthine (Hyx^-), and uracil (Ura^-) or for combinations of these requirements. Strains with no specific requirements are regarded as prototrophic (Proto).

Plasmid profile analysis

Plasmids were identified following extraction based on a procedure described by Kado and Liu[12]. Control strains with plasmids of known molecular weights were included as size markers in agarose gel electrophoresis.

Results

Auxotypes

Of the 60 isolates examined, 25 were PPNG strains. The 25 PPNG strains could be resolved into four auxotypes and the 35 non-PPNG strains were differentiated into five auxotypes. The Pro^- auxotype predominated in both PPNG (in 14, 56%) and non-PPNG strains (in 17, 42·5%), followed by prototrophic (Zero auxotype) strains (in 7 PPNG, 28% and in 13 non-PPNG, 32·5%). The Pro^- Arg^- auxotype was represented by three PPNG and three non-PPNG strains.

Serogroups and serovars

Most of the PPNG (20, 80%) and non-PPNG (33, 94·3%) strains belonged to the WII/III serogroups. Strains belonging to the WI serogroup was further differentiated into two serovars and the WII/III serogroup was represented by 11 serovars. The serovar Aedih predominated amongst the WI serogroup of both PPNG and non-PPNG strains. Only one other strain typed as serovar Aed was found in the WI serogroup. The predominant serovars identified amongst the WII serogroup strains were Bacjk (22·4%), Bajk (17·2%), Bagjk (12%) and Beghjk (10·3%). The PPNG strains could be resolved into 11 different serovars, four auxotypes but 14 different auxotype/serovar classes. The 35 non-PPNG strains belonged to 12 different serovars, five auxotypes and were subtyped into 22 different auxotype/serovar classes.

Plasmid profiles

The 35 non-PPNG strains were divided into three main groups based on the plasmid profiles generated. Two profiles were observed amongst the 25 PPNG isolates. The cryptic plasmid of 2·6 MDa was present in both PPNG and non-PPNG isolates. Non-PPNG strains (27, 77·1% of the total non-producing) with plasmid profile I carried only 2·6 MDa cryptic plasmid whilst five strains (14·3% of total non-PPNG) had the conjugative plasmid of 24·5 MDa in conjunction with the 2·6 MDa plasmid. Non-PPNG strains (3, 8·6%) displaying plasmid profile III were found to carry three plasmids of M.wt. 2·6 MDa, 7·8 MDa and 24·5 MDa (table 1).

Two plasmid profiles were evident amongst the 25 PPNG strains. Group I PPNG strains (16, 64% of the total PPNG) carried the 2·6 MDa plasmid and the 4·4 MDa penicillinase plasmid (Asian type). Group II PPNG strains (9, 36%) harboured the 24·5

Table 1 Plasmid profile of PPNG and non-PPNG strains

Non-PPNG strains (n = 35)		*PPNG strains (n = 25)*	
Plasmid profile (M. Wt. in MDa)	*No of strains*	*Plasmid profile (M. Wt. in MDa)*	*No of strains*
I 2·6	27	I 2·6 + 4·4	16
II 2·6 + 24·5	5	II 2·6 + 4·4 + 24·5	9
III 2·6 + 7·8 + 24·5	3		

MDa = megadaltons.

Table 2 Relation between plasmid profile, auxotype, serogroup and serovar of 35 non-PPNG strains

Plasmid profile	*Auxotype*	*Serogroup*	*Serovar*	*No of strains*
2·6	Proto	WI	Aedih	1
2·6	Pro⁻ Met⁻	WI	Aedih	1
2·6	Pro⁻	WII/III	Bak	1
2·6	Pro⁻	WII/III	Back	3
2·6	Pro⁻	WII/III	Bajk	3
2·6	Pro⁻	WII/III	Bcgk	2
2·6	Pro⁻	WII/III	Bacjk	4
2·6	Pro⁻	WII/III	Bcgjk	2
2·6	Pro⁻	WII/III	Beghjk	1
2·6	Pro⁻Hyx⁻	WII/III	Bacjk	1
2·6	Pro⁻Arg⁻	WII/III	Bacjk	1
2·6	Proto	WII/III	Bak	1
2·6	Proto	WII/III	Back	1
2·6	Proto	WII/III	Bajk	1
2·6	Proto	WII/III	Bhk	1
2·6	Proto	WII/III	Beghjk	3
2·6 + 24·5	Pro⁻Arg⁻	WII/III	Bajk	1
2·6 + 24·5	Proto	WII/III	Bajk	1
2·6 + 24·5	Proto	WII/III	Bck	1
2·6 + 24·5	Proto	WII/III	Bcegk	1
2·6 + 24·5	Proto	WII/III	Bcegjk	1
2·6 + 7·8 + 24·5	Pro⁻	WII/III	Bajk	1
2·6 + 7·8 + 24·5	Pro⁻Arg⁻	WII/III	Bcgjk	1
2·6 + 7·8 + 24·5	Proto	WII/III	Bcgk	1

MDa conjugative plasmid in addition to the 2·6 MDa and 4·4 MDa plasmids. The 3·2 MDa (African type) penicillinase plasmid was not detected in both PPNG and non-PPNG.

Relation between auxotypes, serogroups, serovars and plasmid profiles

Analysis of non-PPNG strains

Table 2 presents the relation between auxotypes, serogroups, serovars and plasmid profiles of the 35 non-PPNG strains tested. Majority (87·5%) of the strains carrying the 2·6 MDa cryptic plasmid belonged to the WII/III serogroup. Seven of these were of auxotype Pro⁻ whilst five were prototrophic (non-requiring). Some strains with identical plasmid profile, auxotype and serogroup were distinguishable only by their serovars. Seven serovars were observed amongst the Pro⁻, WII/III serogroup strains that carried the 2·6 MDa plasmid. Similarly, five Prototrophic WII/III serogroup strains harbouring the 2·6 MDa plasmid were found to be comprised of five serovars. Two strains that had identical plasmid profile (2·6 MDa), serogroup (WI) and serovar (Aedih) were found to be of different auxotypes. This was similarly observed for two other strains of serogroup WII/III, serovar Bacjk that harboured the same 2·6 MDa plasmid.

Of the five strains harbouring the 2·6 MDa cryptic plasmid and the 24·5 MDa transfer plasmid, four were prototrophic (non-requiring) and one required proline and arginine for growth. The four wild type strains could only be differentiated by their serovar differences. Two serogroup WII/III strains (of serovar Bajk) carrying the 2·6 MDa and 7·8 MDa plasmids were found to be differentiated only by their auxotypes. Three serogroup WII/III strains har-

Table 3 Relation between plasmid profile, auxotype, serogroup and serovar of 25 PPNG strains

Plasmid profile	*Auxotype*	*Serogroup*	*Serovar*	*No of strains*
2·6 + 4·4	Pro⁻	WI	Aed	1
2·6 + 4·4	Pro⁻	WII/III	Bck	1
2·6 + 4·4	Pro⁻	WII/III	Bajk	2
2·6 + 4·4	Pro⁻	WII/III	Bacjk	4
2·6 + 4·4	Pro⁻	WII/III	Bcgjk	3
2·6 + 4·4	Pro⁻	WII/III	Beghjk	1
2·6 + 4·4	Pro⁻Met⁻	WII/III	Bcgjk	1
2·6 + 4·4	Pro⁻Arg⁻	WII/III	Bcgk	1
2·6 + 4·4	Pro⁻Arg⁻	WII/III	Bcegjk	1
2·6 + 4·4	Proto	WII/III	Bacjk	1
2·6 + 4·4 + 24·5	Pro⁻	WI	Aedih	3
2·6 + 4·4 + 24·5	Proto	WI	Aedih	1
2·6 + 4·4 + 24·5	Pro⁻Arg⁻	WII/III	Bcegjk	1
2·6 + 4·4 + 24·5	Proto	WII/III	Bak	1
2·6 + 4·4 + 24·5	Proto	WII/III	Back	1
2·6 + 4·4 + 24·5	Proto	WII/III	Bacjk	2

bouring the 7·8 MDa plasmid in conjunction with the 2·6 MDa and 24·5 MDa plasmids were represented by three auxotypes and serovars.

Analysis of PPNG strains

Of the 16 PPNG strains harbouring the 2·6 MDa and the 4·4 MDa (penicillinase) plasmids, only one strain belonged to serogroup WI. The 15 WII/III serogroup strains could be further differentiated by auxotype and serovar differences. These strains were represented by five auxotypes and seven serovars (table 3).

The nine PPNG strains carrying the 2·6 MDa, 4·4 MDa (penicillinase) and 24·5 MDa plasmids were distributed into two serogroups, three auxotypes and five serovars. Of the four serogroup WI strains exhibiting the same plasmid profile and serovar pattern, one was found to be prototrophic (non-requiring) whilst the other three were Pro$^-$. Serovar was the only notable difference observed amongst four strains displaying the same serogroup (WII/III), plasmid profile (2·6, 4·4, 24·5) and auxotype (Proto).

Discussion

Phenotypic characterisation of *N gonorrhoeae* isolates by auxotype, plasmid profile and serovar analysis has provided accurate definition of gonococcal populations. Epidemiological data gathered using this combined approach readily presents a baseline definition of gonococcal strains circulating in any community. The present study provides a detailed characterisation of *N gonorrhoeae* strains isolated from Singapore in 1984 and the data will be useful for local prospective study of gonococcal infection and for monitoring spread of these strains to other countries.

Characterisation of both PPNG and non-PPNG strains on the basis of auxotypes, plasmid profiles and serovars indicated the heterogeneity of strains comprising each group. The 25 PPNG strains were separated into 16 different combinations whilst 24 different classes were seen amongst the 35 non-PPNG strains. Conclusions drawn regarding the diversity of our local gonococcal populations would have been quite different if we had relied on either auxotype and plasmid profile analysis or auxotype and serovar classification.

Our results extend some previous studies[3 10 13] regarding the distribution of auxotypes, serogroups, serovars and plasmid profiles. The Pro$^-$ and prototrophic (proto, non-requiring) strains were seen as the predominant nutritional types amongst both PPNG (Pro$^-$, 52%; Proto, 24%) and non-PPNG (Pro$^-$, 48·5%; Proto, 37·1%) strains. This observation differed from an earlier study[10] which showed prototrophic and Pro$^-$ auxotype as the preponderant nutritional groups amongst PPNG and non-PPNG strains respectively. In the present study, Pro$^-$ strains were predominant amongst both PPNG and non-PPNG strains.

With the exception of five serogroup WI strains, the rest (55 strains) belonged to the WII/III serogroup. The serovar Aedih dominated among WI PPNG and WI non-PPNG strains. Our results agree with previous observations[10] that serovar Aedih predominates amongst the WI serogroup strains from South East Asia. As our sample size of serogroup WI strains was small, we did not come across unusual serovars such as Aed, Ad, Afe and Af serovars as was reported earlier[3]. Eleven different serovars were observed among the WII/III non-PPNG strains. Serovars Bajk (20%), Bacjk (17%), Back (11·4%) and Beghjk (11·4%) were the major serovars observed. WII/III PPNG strains were represented by nine serovars and the predominant serovars were Bacjk (28%) and Bcgjk (16%).

The 2·6 MDa cryptic plasmid was found to be present in all the 60 strains examined in this study. It is interesting to note the presence of the 7·8 MDa cryptic plasmid in three non-PPNG strains. Restriction digests of plasmid DNA from these three strains with *Hinf*I and *Ava*II/*Hinf*I produced identical profiles to the 2·6 MDa plasmid DNA similarly digested. The data suggested that the 7·8 MDa plasmids are identical to the 2·6 MDa plasmid and are concatemers of the 2·6 MDa plasmid as described previously.[14] All 35 non-PPNG strains were found to carry the 2·6 MDa cryptic plasmid either alone (45·6%) or in conjunction with the 24·5 MDa transfer plasmid (22·9%). The PPNG strains (41·6% of total) were found to harbour the Asian type resistance plasmid (4·4 MDa) either in conjunction with the 2·6 MDa plasmid or the 24·5 MDa transfer plasmid plus the 2·6 MDa plasmid.

Strains carrying the 4·4 MDa B-lactamase plasmid in conjunction with the 2·6 MDa plasmid were mainly Pro$^-$ whilst those carrying the 24·5 MDa plasmids were mainly wild-type strains. Diversity of serovars was observed among Pro$^-$ strains containing both 2·6 MDa and 4·4 MDa plasmids. This was similarly observed among wild-type strains carrying the 24·5 MDa transfer plasmid in conjunction with the 2·6 MDa and 4·4 MDa plasmids. The presence of the same plasmid combinations (2·6 MDa + 4·4 MDa + 24·5 MDa) in four Proto strains that exhibit serovar diversity suggests that the transfer of these plasmids by either conjugation or transformation has played an important role in their spread. No PPNG isolates was found to carry the 3·2 MDa "African" B-lactamase plasmid or the 3·05 MDa "Toronto" plasmid. The 25·2 MDa tet M-conjugative plasmid was similarly not detected.

The combined approach of applying auxotyping, plasmid profile analysis and serological classification has enabled us to characterise the gonococcal popula-

tion in Singapore. The data clearly show that *N gonorrhoeae* strains isolated here differed from those that were endogenous in Canada,[3] Africa[1] and Greece.[4] Strains isolated from these three regions tend to belong to serogroup WI whereas serogroup WII/III strains were predominant in our study. Predominant auxotypes such as AHU^- and PCU^- typical of Canadian isolates were not detected in our samples.

Characteristic biological differences found between gonococcal strains isolated from different geographical areas of the world are useful for tracing exportation of strains. Such differences have also led to a better assessment of the efficacy of antibiotic treatment as strains could be readily differentiated prior to and after treatment. We found in this study serovar diversity has been the most useful biological difference for strain differentiation.

Address for correspondence: Dr C L Poh, Department of Microbiology, National University of Singapore, Lower Kent Ridge Road, Singapore 0511.

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